EXHIBIT

StressGen Biotechnologies Corp.
Molecular Biology Projects

Name of Construct: pTrc65/LF(4N+1C)
Start Date:
Lab Book#: AG-5, Page 27
Primary Researcher: Bill Wu & Cor Turnnir

Description:

4 repeats of P1A epitope (4 x LPYLGWLVF) was fused to the N-terminus of BCG Hsp65 gene in pTrc65/LF. The resulting plasmid expresses Hsp65 fused at its N-terminus with 4 repeats of P1A and at its C-terminus with 1 repeat of P1A

Cloning Procedure:

Two oligos coding for 5 repeats of P1A and a thrombin cleavage site were systhesized. Sense strand: cat ggc tct gcc gta tct ggg ctg gct ggt gtt cct gcc gta tct ggg ctg gct ggt gtt cct gcc gta tct ggg ctg gct ggt gtt cct gcc gta tct ggg ctg gct ggt gtt cct ggt tcc gcg tgg atc. Antisense strand: cat gga tcc acg cgg aac cag gaa cac cag cca gcc cag ata cgg cag gaa cac cag cca gcc cag ata cgg cag gaa cac cag cca gcc cag ata cgg cag gaa cac cag cca gcc cag ata cgg cag agc.

These two oligos which contain NcoI overhang on both ends were annealled and re-amplified with W066 (cta agt gcc atg gct ctg ccg tat ctg ggc) and W067 (agt cta agc cat gga tcc agc cgg aac cag). The PCR product was digested with NcoI, purified and ligated to pTrc65/LF DNA which had been previously digested with *Nco* I. The ligation mixture was transformed into *DH5α* and putative clones containing *P1A* N-terminal insert in the right orientation was selected by miniprep screening . The PCR product that was successfully amplified and inserted contained 4 repeats of P1A instead of 5 repeats. Maxiprep DNA was prepared and confirmed by diagnostic restriction digestion. Plasmid DNA was transformed into appropriate E. coli strain for expression analysis. The location of the fusion gene within the construct is illustrated on the following plasmid map.

Prepared by: _____ Date: _____

— Larry's sample.

① STD. BG465, 97. 98. 99. 100. 101. 102. 103. 105. 106 107. 108 p815⊖.

② BG465, STD. 110. 111. 112. 113 114. 115. 116 117. 118. 119. 120. p815⊖

all 5ul loaded except p815⊖ (20ul).

— Set up PCR.

	①	②	③	④	⑤	⑥
WO6U (new)	2	2	2	2	2	/
WO6t (old).	2	2	2	2	2	/
10× Buffer	10	10	10	10	10	10
10 mM dNTP	2	2	2	2	2	2
WO66	1.0	/	1.0	1.0	/	1.0
WO67	/	1.0	1.0	1.0	/	1.0
pfu pol.	0.5	0.5	0.5	0.5	0.5	0.5
SDW	82.5	82.5	81.5	81.5	83.5	85.5

Program #74. (96°C. 68°C. 72°C)

Continued on Page

Read and Understood By

Signed Date Signed Date

REQUEST FOR DNA SYNTHESIS

P.O. Number _____

Order Date _____ Date Required _____

Dalton Chemical Laboratories Inc.
119 Farquharson Bldg., 4700 Keele St.
North York, Ontario, M3J 1P3
Tel: (416) 736-5394, Fax: (416) 736-5846
Attn: Peter Pekos

Customer Information

Dr. Bill Wu. Streatgen
Name

#120-4243 Glanford Ave.
Address

Victoria B.C. V8Z 4B9
City Province Post/Zip

Tel. (604) 744-2811
Telephone Fax (604) 744-2877

Billing Information P.O. Attached _____

Streatgen.
Billing Name

#120-4243 Glanford Ave.
Billing Address

Victoria B.C. V8Z 4B
Billing City Province Post/Zip

Special Instructions

Desalted DNA oligo without HPLC or PAGE purificat

No. of OD units required 5-10

Sequence 1 Sequence Name WC64 (159 mer)

5' (P) CAT GGC TCT GCC GTA TCT GGG CTG GCT
→ GGT GTT CCT GCC GTA TCT GGG CTG GCT
→ GGT GTT CCT GCC GTA TCT GGG CTG GCT
→ GGT GTT CCT GCC GTA TCT GGG CTG GCT
→ GGT GTT CCT GCC GTA TCT GGG CTG GCT
→ GGT GTT CCT GGT TCC GCG TGG ATC ...
→ ...
→ ...
→ ...
→ ...

5 repeats for maltose binding peptide (LF). w/ thrombin cleavage site. No 1 at both ends, also (P)

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Special Instructions

Desalted DNA oligo without HPLC or PAGE purification

No. of OD units required 5-10

Sequence 1 Sequence Name W065 (159 mer)

5' (P) CAT GGA TCC ACG CGG AAC CAG, GAA CAC
CAG CCA GCC CAG ATA CGG CAG, GAA CAC
CAG CCA GCC CAG ATA CGG CAG, GAA CAC
CAG CCA GCC CAG ATA CGG CAG, GAA CAC
CAG CCA GCC CAG ATA CGG CAG, GAA CAC

5' CAG CCA GCC CAG ATA CGG CAG AGC ...

5 repeats for mass tomb. peptide (LF). oligo (2).

STRESSGEN BIOTECHNOLOGIES CORP.
#120 - 4243 Glanford Avenue, Victoria, BC, Canada V8Z 4B9 Tel: (604)744-2811 Fax: (604)744-2877

OLIGO REQUISITION FORM

Required by Bill Wu Month ____ Day ____ Year ____

Sequence (PLEASE PRINT CLEARLY in triplet form, direction 5' to 3')

5' AGT CTA AAC CAT GGA TCC ACG CGG AAC CAG 3'
1 30
Nco I
31 60
61 90
91 120
5' 121 150 3'

Sequence name W067 (name that means something to you)

Sequence length 30 mer

Comments: PCR primer ② For LFS oligo. Reverse.

HPLC purified, lyophilized oligo ____ ug= ____ pmols SYNTHESIS# ____

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OLIGO REQUISITION FORM

Required by Bill Wu Month ____ Day ____ Year ____

Sequence (PLEASE PRINT CLEARLY in triplet form, direction 5' to 3')

5' CTA AGT GCC ATG GCT CTG CCG TAT CTG GGC 3'
1 30
Nco I
31 60
61 90
91 120
5' 121 150 3'

Sequence name W066 (name that means something to you)

Sequence length 30 mer

Comments: FCR Primer ① For UFS oligo. Forward

HPLC purified, lyophilized oligo ____ ug= ____ pmols SYNTHESIS#____